DETERMINING HOW FRESHWATER COPEPODS FOLLOW PLANAR DEXTRAN TRAILS

A Thesis
Presented to
The Academic Faculty

by

Madison Young

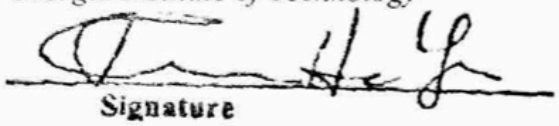
In Partial Fulfillment
of the Requirements for the Degree
B.S. in Biology with the research option in the
School of Biological Sciences

Georgia Institute of Technology
May 2017

DETERMINING HOW FRESHWATER COPEPODS FOLLOW PLANAR DEXTRAN TRAILS

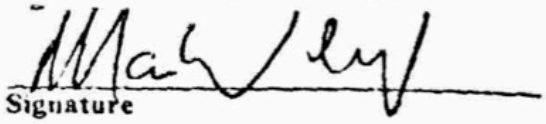
Approved by:

Dr. Jeannette Yen, Advisor
School of Biology
Georgia Institute of Technology



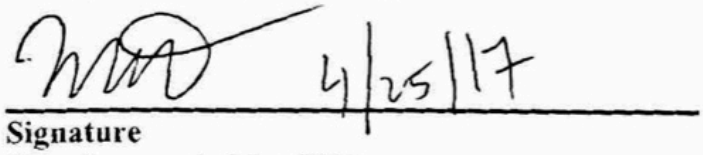
Signature

Dr. Marc Weissburg
School of Biology
Georgia Institute of Technology



Signature

Dr. Michael Goodisman
School of Biology
Georgia Institute of Technology



Signature
Date Approved: May 2017

ACKNOWLEDGEMENTS

I wish to thank Dr. Jeannette Yen for the opportunity to begin working in her research lab as a sophomore at Georgia Tech. Without her guidance and support I would not have been able to complete this thesis and the research option. I would also like to thank Larisa Pender-Healy for being my mentor for two years and for teaching me the foundational skills necessary to complete my project. Additionally, I would like to thank Andrew Sikafoose for designing and producing some of the tools necessary for this experiment and the undergraduate research students in the Yen lab for assisting and supporting me as issues arose throughout the semester.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, ABBREVIATIONS, AND TERMINOLOGY

Encounter- when a male copepod disturbs a flowing trail without reorienting its body or truly noticing and following the trail

Follow- when a male copepod encounters a flowing trail and then reorients its body to be parallel with the trail and then disturbs the trail for a certain distance leaving a distinct residue (pattern) behind it

Strouhal's number- a way of determining the efficiency of swimming and flying animals, and it is found by dividing the stroke frequency (f) and the amplitude (A) by the forward swimming speed (U). The full equation reads St=fA/U, and ultimately measures the propulsive efficiency of animals over a certain period of time.

SUMMARY

In this study two species of freshwater copepods, *Hesperodiaptomus shoshone (H. shoshone),* and *Hesperodiaptomus arcticus (H. arcticus)* will be used to determine how some species of freshwater copepods interact with planar dextran trails. When a copepod swims through water, hydrodynamic disturbances with a variety of structures are created; some are vortical, planar, or laminar. Initial studies show that these copepods avidly follow laminar trails in an upstream fashion [Pender Healy]. However, when copepods execute turns or fast swimming, vortices are created. When copepods execute slow turns, planar wakes are formed. The direction of flow in the wakes and the location of the wakes provide information on the location of the copepod that is generating that wake. The intent of this research is to determine if the signals in the wakes can lead the following copepod to the source of the disturbance. Hence, all analyses focused on events where a copepod responded to the signal. Responses include reorientation or angle of entrance, time spent in trail, preference for a particular trail width and edge following behavior [Strouhal number]. The goal is to understand more about their how they sense and respond to changes in their environment and it is hypothesized that both species will interact more with the wide trail and that *H. arcticus* will spend more time in the trail and enter at a greater angle. It is also hypothesized that both species adhere to ideal Strouhal values. To test this, two dextran (a polysaccharide) trails will be simultaneously dropped into a tank containing the copepods. One of the trails will be 2mm and the other will be 4mm. It is expected that the copepod will wobble or traverse the trail to contact the edges; edge following enables the copepod to stay on track. Alternatively, the copepod may follow the center of the trail where the flow is the fastest and therefore is relying on

flow speed rather than the shear found in the edge of the trail. Analyses of the location of the follower relative to the edge versus the center of the trail can assess whether the copepod is sensing flow shear versus flow speed. A MatLab script will be used to find more detailed information (Figures 11-22).

Both species prefer to follow the wider trail (Figure 5 and 6), and *H. arcticus* spend more time in the trail than *H. shoshone*. *Hesperodiaptomus shoshone* followed the 4mm trail eighteen times for an average of 2.14 seconds and the 2mm trail five times for an average of 1.36 seconds. The number of encounters was also determined, which confirms that the proportion of encounters resulting in follows is much higher for the 4mm trial than the 2mm trial in both species (Table 3). These data illustrates statistically significant results ($p<0.05$) that *H. shoshone* prefer to follow the wider trail and follow for longer periods of time compared to the smaller one. These results were compared to the results for *H. arcticus,* which followed the 4mm trail twelve times for an average of 5.08 seconds (Figure 6). This comparison between both species on the 4mm trail confirmed that *H. arctius* spend more time in the trail than *H. shoshone* ($p<0.05$). The average angles of entrance were 29.16° for *H. shoshone* on the 4mm trail and 21.08° on the 2mm trail, and 39.69° for *H. arcticus* on the 4mm trail (Table 2). When compared, the results demonstrated that *H. arcticus* enters the trail at a greater angle than *H. shoshone* on both the 2mm and 4mm trails ($p<0.05$). There was not enough of the wobbling behavior shown to find Strouhal Values at this time.

CHAPTER 1

INTRODUCTION

Understanding how certain organisms find and respond to chemical cues in the water is important to understanding how they interact with other organisms in their environment and function in their ecological niche as a whole (Amos et al 2016). Behavioral Ecology is a field that focuses on this aspect. The Yen lab at Georgia Institute of Technology concentrates on aquatic chemical ecology and Biologically Inspired Design, and currently a great deal of research is taking place based upon observation of the behavior of freshwater copepods, a planktonic organism. Previous work in the Georgia Institute of Technology's Biology department has focused on one species of copepod in particular - *Hesperodiaptomus shoshone* (Pender-Healy et al 2012). Until this point, previous research has focused on how copepods respond to chemical cues in the water by simulating mating, feeding, or predation events. Hydrodynamic cues in nature are used for many behaviors including locating a mate, identifying food, communicating, and spermatophore placement (Frederick et al 2015). Planktonic organisms, such as copepods, are studied in order to understand how they respond to various hydrodynamic cues. Tracking and finding resources is the most important task for a copepod, and their minimal sensory organisms make their survival fascinating.

Copepods are among the most prevalent metazoans in the ocean and other aquatic environments and are important in many fields of study. Planktonic organisms contribute to maintaining tropic dynamics and a thriving ecosystem (Wang et al 2012). In this study their swimming patterns were studied to help with these applications. Copepods can either move via oscillatory movements or by "jumping" when they sense a signal.

Chitinous appendages like swimming legs and tail create the movement and propel the copepod in the desired direction (Wang et al 2012). When the organism jumps, it can increase its swimming speed by up to five times its normal velocity, which is a component that will be reviewed in the future based upon the results of this study if the data are appropriate. The jumping pattern assists with feeding efficiency, and is triggered in response to hydrochemical signals such as changes in density or flow disturbances. This is why planar dextran trails trigger a response on *H. arcticus* and *H. shoshone*.

Hesperodiaptomus shoshone and *H. arcticus* are both members of the genus *Hesperodiaptomus light*, which is a diverse group of freshwater copepods found in North America and Central America in high-altitude alpine lakes (Frederick et al 2015). Typically *H. shoshone* is found in more southern locations. Within this genus, the copepods are both predatory and herbivorous species (Marszalek et al 2008). *Hesperodiaptomus shoshone* have a mean body size of 2.5mm and a mean antennae span of 4mm (Yen et al 2011), while *H. arcticus* are smaller and have a body size of about 1.5mm and antennae span of about 2mm. Both species are large compared to other species of freshwater copepods, and inhabit fishless, high-altitude lakes. These species are both large and bright in coloration, making them susceptible to predation, which is why they are found in fishless lakes (Fischer et al 2011). Copepods play an important role in maintaining the ecosystem in the lake as a whole and this study clarifies how they follow and respond to hydrodynamic cues. In addition to helping the ecosystem function, they serve as a food source for other organisms. Unlike phytoplankton, zooplankton do not make their own food and therefore must eat algae or *Daphnia*, another species of

crustacean. The ecosystem of the lake is managed and the environment remains constant throughout climate changes as the algae is eaten.

Within the experiments conducted sometimes a behavior known as “casting” or “wobbling” is noted. This occurs when the animal has found and followed the trail, but wobbles back and forth between the edges without leaving instead of simply following the trail straight up in the center. This could be inefficient, but it could also be a vital component to understanding how the copepod moves. Tracking this behavior more closely and finding Strouhal’s number for *H. shoshone* and *H. arcticus*, may provide a reason for certain behaviors. An ideal Strouhal number is between 0.2 and 0.4 (Taylor et al 2006). If the results of this experiment show that *H. shoshone* and *H. arcticus* exhibit casting and adhere to ideal Strouhal values, this field will be closer to stating that all fresh water copepods show casting in trails while maintaining a high efficiency as movement occurs in the water. In the Yen lab, a Strouhal number calculation has not yet been done, so there is potential for expanding this experiment to copepod species other than *H. arcticus* and *H. shoshone* if the results are significant.

CHAPTER 2

LITERATURE REVIEW

Behavioral Ecology is a subject area centered on how organisms interact with all components their environment. All organisms face pressure to find suitable mates, use their resources to obtain adequate amounts of nutrients, and meet fitness requirements. One component of Behavioral Ecology is understanding how various organisms find and respond to chemical, environmental, and social cues. One of the areas the Yen lab at Georgia Institute of Technology specializes in is trying to understand the behavior of freshwater copepods, a planktonic organism. Copepods are a dominating mesozooplankton group and are possibly the most abundant metazoans on the earth, which could be due to their profound ability to detect hydrodynamic disturbances to their environment (Kiorboe et al. 2010). Once they sense these disturbances which can either be food or mating trails that they want to follow, or predator disturbances the copepods want to avoid, they can act accordingly and change their path at very high velocities for their size (Wang et al 2012). There are also many species of marine copepods, but the research conducted in this study focused only on freshwater species in order to determine if there are behavioral similarities amongst many species of freshwater copepods.

Previous work has been done in the Georgia Institute of Technology Biology department on *H. shoshone,* a species of freshwater copepod. Sometimes *H. shoshone* shows a behavior known as "casting" where once they have found and followed the trail, they wobble back and forth between the edges of it without leaving (Pender-Healy et al. 2012). At this point in the experimental process, the casting behavior has not been quantified. Experiments have also been conducted with other species including *H.*

arcticus, but there is much less information about these two species. *H. arcticus* shows more movement and casting inside the trail than *H. shoshone*, but more data needs to be collected to make significant conclusions. *Hesperodiaptomus shoshone* are much larger than *H. arcticus*. The primary consequence of a difference in size is the separation of sensory setae along the antennule. For a smaller copepod, the setae are more closely spaced so that smaller hydrodynamic features can be detected. The increased movement of the smaller copepods may indicate it is detecting finer fluid deformations in the trail. Most previous research that has been done up to this point has been on how copepods respond to chemical cues in the water from simulating mating, feeding, or predation events. Chemical cues in nature are used for many behaviors including locating a mate, identifying food, communicating, and spermatophore placement (Frederick et al 2015).

In one scientific paper based on research done in the Yen Lab, Pender-Healy et al. discusses the hydrodynamic cues aquatic animals can sense. Ultimately, hydrodynamic cues are used to sense predators or potential mates. When the copepod swims though the water, the fluid is displaced and disrupted, which causes a wake to follow the organism. This wake is the hydrodynamic cue that the copepods sense or feel and respond to accordingly. This thesis is a good starting point for future research because it has a lot of background information on *H. shoshone*, which means that some of the preliminary experiments will not need to be done and future research can be focused on finding Strouhal's number for freshwater copepods. However, this paper reviews the topic of casting without elaborating too much on the impact it has on the copepods swimming efficiency. The goal at the end of the experiments discussed in this thesis is to fill in some of the gaps and answer remaining questions.

To find Strouhal's number, low magnification videos will be tracked to determine concrete numbers that can ideally be matched with observations. The tracking will be done using a MatLab computer program that records various points in a coordinate system in the videos obtained from experiments. From these points, different values such as angle variation, velocity, and position relative to the trail and other copepods can be found. Tracking this behavior more closely may give insight into how closely freshwater copepods adhere to an ideal Strouhal's number. An ideal Strouhal's number was found to be between 0.2 and 0.4 (Taylor et al 2006). Quantifying Strouhal's number may determine the reason behind the casting behavior. If the results of this experiment show that *H. shoshone* and *H. arcticus* exhibit casting and adhere to ideal Strouhal values, this field will be closer to being able to state that all fresh water copepods show casting in response to sensors while maintaining a high efficiency in regards to how they move in the water to find mates and escape predators.

Taylor et al's paper on Strouhal's number states that the animals tested in the experiment included different species of fish, bats, insects, and birds (Taylor et al 2006). Even though this is a well-written scientific paper, it leaves many questions unanswered. One question in particular is that it does not elaborate on what happens to an animal that is outside of an ideal Strouhal number. Are they simply less fit compared to other organisms, or do they suffer a harsher fate? Additionally, there is not much range in the size of organisms tested. The research the Yen Lab is conducting will hopefully fill some of the size so the scientific community will be able to better explain the behavior of swimming and flying animals. Another study centered on copepods found that they had a high power stroke efficiency that corresponded to peak propulsion efficiency while being

inside the ideal range for Strouhal's number (Kiorboe et al 2010). This study did not specify what species of copepod was used, but it further confirmed that copepods are fascinating aquatic organisms.

The goal of the current study is to determine how common this behavior is amongst freshwater copepods, and the first step is to run experiments using *H. shoshone* and *H. arcticus*. There is potential for applying this experiment to other species of copepods such as aquatic ones if the results are promising. Assuming the results of this experiment provide a good foundation, this experiment will be important in determining how copepods of all species fit into their ecological niche and if their behavioral traits are consistent with other species of swimming animals. Additionally, this research will help fill some gaps in Taylor et al's paper on Strouhal's number in flying and swimming organisms and answer remaining questions about the mating, feeding, and social efficiency of *H. shoshone*.

CHAPTER 3

METHODS AND MATERIALS

Animal Collection and Care

Hesperodiaptomus shoshone copepods were collected from a freshwater lake in Montana, Dissertation Lake, using a plankton net. Usually this process takes several days. However, this year, due to the multitude of copepods in the lake, the collection process only took one day. It was vital to collect the copepods in the beginning of August because the lake freezes in September and does not thaw out until July. The collection methods were the same for *H. actcitus* and the experimental setup was also identical.

The copepods were then shipped to the Yen Lab in Atlanta in insulated containers set on ice. Once the copepods arrive, they were immediately transferred into larger buckets that have artificial lake water with the same pH as their natural habitat; this year that pH was 6.5. The copepods must be immediately transferred to containers that have identical conditions to what they came from in Montana. The copepods were kept in a cold room where the temperature is a constant 12°C, identical to their natural habitat. Next, the copepods were sorted so that the males and females are separated. The males can be identified because they have one geniculated antennae that is slightly larger than the other antennae (Reid et al 2010). The females have two identical antennas and an egg sack attached to them. On the male copepods, the geniculated antenna is used to grab females just prior to mating. Since *H. shoshone* and *H. arcticus* are larger species of copepods they can be sorted without a microscope. Once sorted, the copepods were fed concentrated *Daphnia*, a species of plankton. The copepods can eat many different types of plankton or algae, but *Daphnia* was chosen. The experiments begun about one week

after the copepods arrived at Georgia Tech and took place over a span of seven days. The copepods will survive for about four weeks after collection so it was imperative to run experiments as soon as possible in order for the results to be accurate. Once the copepods start to deteriorate in health the experiment can be compromised because they will not be as reactive to trails and their senses will be diminished. For these reasons, the experiments were run as quickly as possible after collection.

Experimental Setup

This experiment used a 3D Schlierien imaging system (Figure 4). Inside the Schlierien imaging system there is a larger outer tank that holds 50 gallons of water and a smaller inner tank that holds 5 gallons. The Schlierien imaging system uses lasers to show differences in density and projects that image onto a camera lens using mirrors. In this experiment only one view was used, but experiments with two views can also be run to produce a 3D image. Planar trails were used in this experiment, so using two views would not be beneficial due to the planar orientation of the copepod when it enters the trail.

In the smaller tank, a water chiller was added so that the water temperature comes to 12°C, the same temperature as the climate the copepods are used to being in. The chiller will stay in the inner tank for the duration of the experiment because if the temperature of the water changes during the experiment, the quality of the image will not be decreased due to dissimilarities in water temperature causing variances in water density. The lasers detect changes in density and will distort the image if the water temperature is not consistent throughout.

The planar trails in this experiment will be made up of a mixture of dextran and artificial lake water (EPA water). To make the dextran solution, 1g of dextran was combined with 10mL of the EPA water with 6.5 pH. This solution was then set to vortex to ensure a homogenous solution was made, and further diluted. To dilute the solution, 1mL of the solution was added to 9 mL of 6.5pH EPA water. This gave the correct density so that the trails can be seen with the laser and the image is clear. After this, 1 drop of blue dye was added to further enhance the clarity of the trail in the 3D Schlierien imaging system. This solution was then put into syringes and placed on a syringe pump that holds one syringe. This solution is also kept at 12°C, using icepacks so that everything in the experiment was at constant temperature. The syringe had a 0.55mm diameter tube attached to it, which leads down into the device that makes the planar trails (Figure 2). The flow rate was set to 0.04ml/min which becomes 2.03 cm s^{-1}. The flow rate was determined by looking at the range of detection for *H. shoshone,* which is about 1.25 ± 0.14 cm s^{-1} normally and 6.8 ± 2.8 cm s^{-1} when a mate is detected (Yen et al 2011). This trail speed was chosen because it is close to the swimming speed of the males so it draws their attention, but not so fast that the trail is too strong for them to follow. The trail speed must be faster than their swimming speed, otherwise they could mistake it for another copepod.

The device that makes the planar trails was built from common items including Legos, a single edge razor blade, zip ties, and aluminum adhesive label (Figure 3). The aluminum adhesive label was put on the razor blade in a manner so that there was a space for the 0.55mm tube to enter and space for the 2mm and 4mm trails to leave. Legos were then used to suspend the blade so that it rested on the edges of the small acrylic tank

while suspending the blade down into the water so the trails could be made. The zip-ties held the blade in place so it did not move and disturb the trails during the experiment.

Running Replicates

Once the water had chilled, 10 male copepods were placed in an even smaller acrylic tank inside the inner tank of the Schlierien imaging system. After this, a planar trail maker was placed in the small acrylic tank and left to settle for fifteen minutes. This step was necessary so the copepods can orient themselves in the tank and the water can settle. The planar trail maker was then attached to a syringe pump, the pump was set to 0.04mL/min, and the trails start to fall down into the small tank. Once the trails run clear, the experiment started.

To start the experiment, a blank DVD was inserted into a DVD player attached to the TV that receives data from the camera in the Schlierien imaging system. Once the "record" button is pressed, the experiment is in progress and runs for thirty minutes. This counts as one replicate. During this replicate, the number of follows and encounters were recorded to get an idea of how the animals were interacting with the trails and if they prefer the smaller 2mm trail or the wider 4mm trail, which were both present due to the device in Figure 2. Preference for a smaller trail may indicate a greater sensitivity to finer hydrodynamic features as might be expected for the smaller copepod *H. arcticus.* Eight replicates were completed in total using 10 different male copepods every time. The whole experimental setup and procedure was identical for all replicates.

Data Analysis

A MatLab computer program, Hendrick's was used to analyze the data and determine if the copepods adhere to ideal Strouhal numbers. In order to use Hendrick's

the videos must be converted using Handbrake, a video editor to set the time stamps on the DVDs. Then the converted files were clipped using SolveignMN, a video splitter. The DVDs were clipped so that the individual follows could be isolated to make the tracking process more efficient. Once the videos were clipped, they were converted to AVI form using Prism Video Converter. They must be in AVI form for Hendrick's to run properly. After the videos were converted, Premier, another computer program was used to enhance the video quality to make the tracking process more exact. The Hendrick's computer program tracks the movements more closely by recording various points in a coordinate system in a frame-by-frame fashion. From these points, which were taken each frame at the rate of 30 frames per second, different values such as angle variation, velocity, and position relative to the trail and other copepods can be found. The head and each end of the antennae were tracked (Figure 1). Additionally, a two-tailed t-test was done to determine if the copepods prefer to follow the 2mm or 4mm trail, which species spends more time in the trail, and if the species enter at different angles. After this, using the data from Matlab Strouhal's number calculation will be done. Since MatLab outputs 'x' and 'y' points, it is possible to determine frequency, amplitude, and forward swimming speed, which are all components necessary (see list of symbols). Time spent in the trail was also measured using the frame-by-frame feature. The angle of entrance was also determined using the equation

$$\theta = 90 - (tan^{-1}\left(\frac{distance\ on\ edge\ of\ trail}{distance\ between\ tail\ and\ edge\ of\ trail}\right)).$$

A MatLab program created by Andrew Sickafoose found the X and Y positions of the head and setae, body angle estimated from the setae location, body angle rate

estimate, measure of position versus filtered position, and filtered velocity of the head and setae, for the *H. arcticus* over all twelve follows in the wide trail (Figures 11-22).

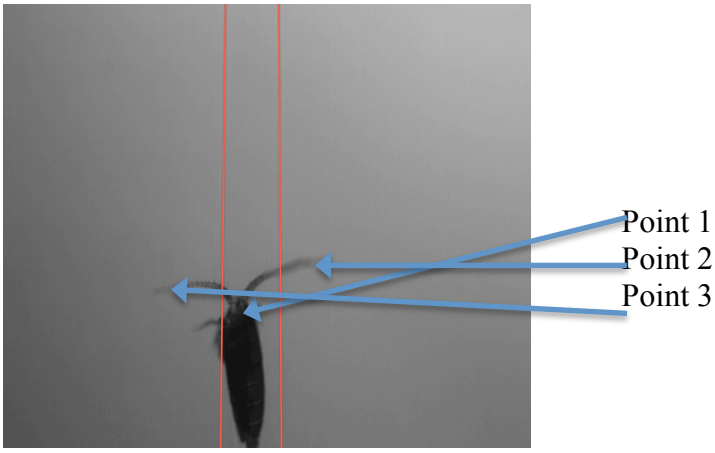


Figure 1: This image shows what points on the copepod will be tracked using Hendricks in order to get the necessary information about position, velocity, and changing body angle

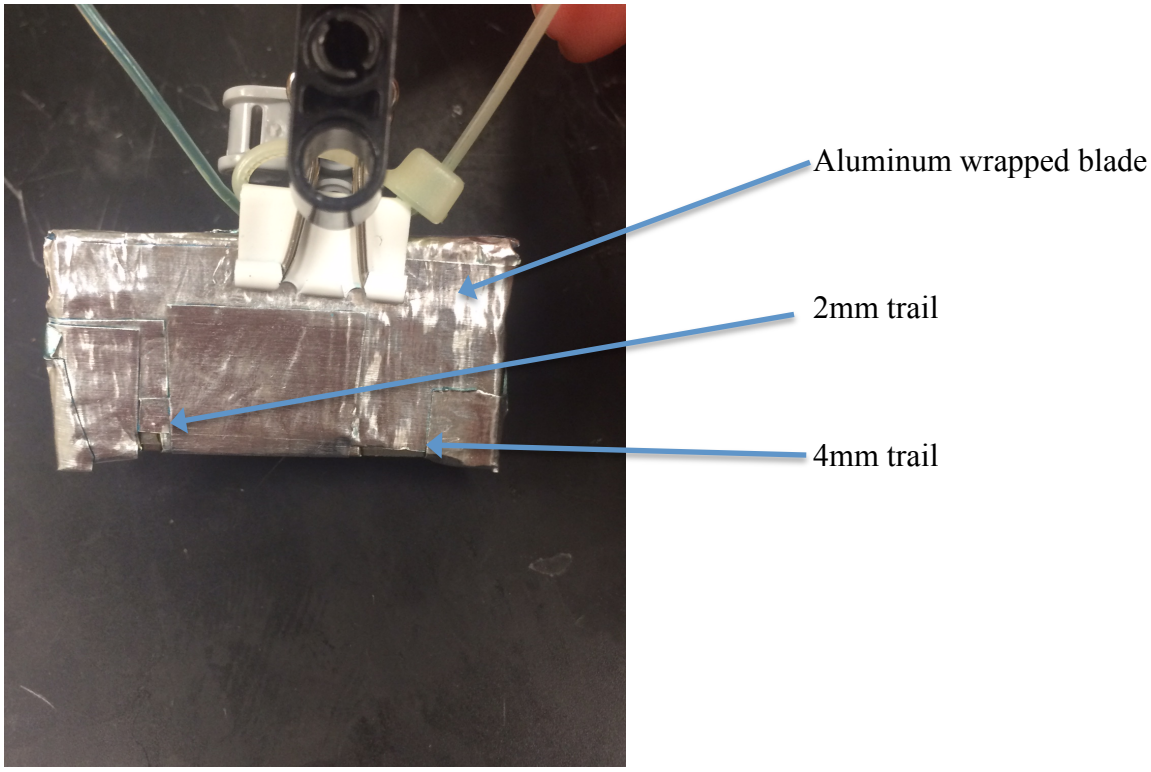


Figure 2: This was the device used to make the planar trails. The opening one the left makes the smaller trail, and the one on the right makes the larger one. The dextran comes in through the top and drops down into the blade to make the trails.

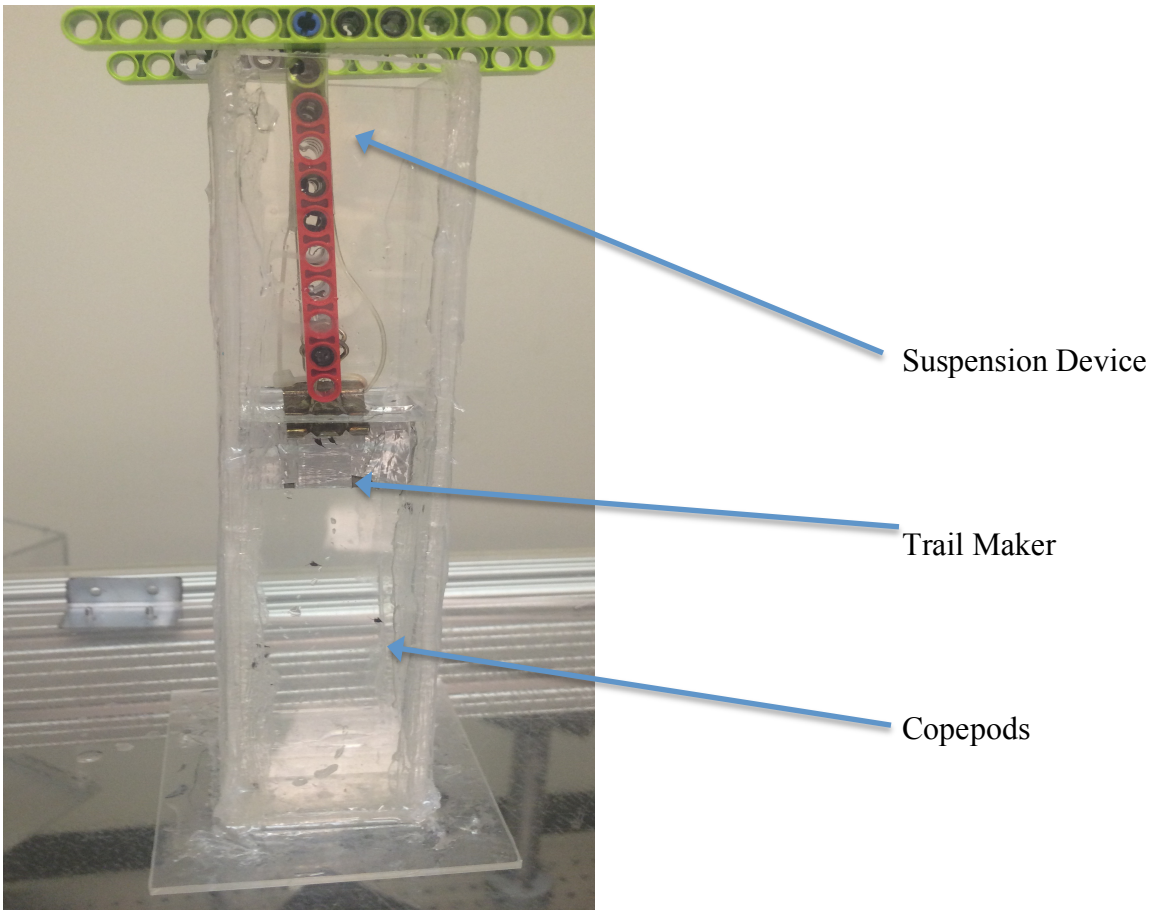


Figure 3: This image depicts the setup of the whole experiment including the trail maker and copepods. This small acrylic tank went inside the smaller tank in the Schlierien.

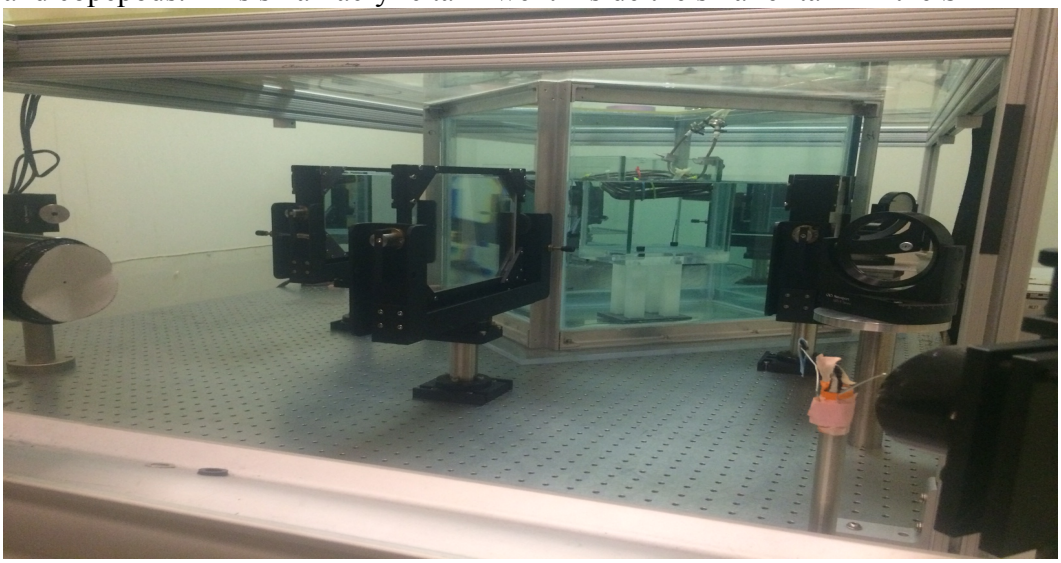


Figure 4: This image shows the 3D Schlieren imaging system used in the Yen laboratory.

CHAPTER 4

RESULTS

The results indicate that *H. shoshone* and *H. arcticus* prefer to interact with the wider 4mm trail and follow it more frequently than the 2mm trail (Table 1). *Hesperodiaptomus arcticus* never followed the 2mm trail; they only encountered it (Table 3). The wobbling behavior cannot be quantified at this point because there are no data to find the frequency. *Hesperodiaptomus shoshone* followed the 4mm trail eighteen times for an average of 2.14 seconds and the 2mm trail five times for an average of 1.36 seconds. The statistical significance of these data was examined using a two-tailed t-test and the results showed that *H. shoshone* prefer to follow the wider trail and follow for longer periods of time compared to the smaller one (Figure 5 and 6) with statistical significance ($p<0.05$). These results were compared to the results for *H. arcticus,* which followed the 4mm trail twelve times for an average of 5.08 seconds. This comparison between both species in the 4mm trail confirmed that *H. arctius* spend more time in the trail than *H. shoshone* ($p<0.05$). The average angle of entrances were 29.16° for *H. shoshone* (standard deviation= 6.08) on the 4mm trail and 21.08° on the 2mm trail (standard deviation =1.69), and 39.69° for *H. arcticus* on the 4mm trail, with a standard deviation of 5.08 (Table 2). When compared the results revealed that *H. arcticus* enters the trail at a greater angle than *H. shoshone* on both the 2mm and 4mm trails ($p<0.05$). Additionally *H. shoshone* prefer to follow the edges of the trail and *H. arcticus* prefer to follow the middle of the trail, potentially because of their smaller antennae span.

The data from MatLab (Figures 11-22) showed that for each of the twelve follows, *H. arcticus* show varying behavior inside the trail but there are consistencies in

the swimming patterns. In most instances the copepod sped up as it entered and exited the trail, this is probably due to the high velocity in the middle of the trail pushing the copepod downstream. The copepods entered and exited the trail on the same side six times, and entered and exited the trail on opposite sides six times. In general, when the copepod enters and exists the trail on the same side the swimming pattern is more consistent and smooth. The changes in velocity are also more consistent when this swimming pattern is followed, the animal increases its' speed when leaving the trail. In figures 11-22 there are five graphs representing different position and velocity measures. The first shows the X and Y positions in millimeters of head and setae of H. *arcticus* copepods while following the trails. The second shows the body angle estimate on the wide trail calculated from the setae position while following the trail. This graph helps determine how much body rotation is present during a follow event. The next graph shows the body angle rate estimate calculated from body angle for *H. arcticus*; time is in the X-axis, and the body angle per second calculation is on the Y-axis. The fourth graph measures the X-position on the X-axis and the Y-position on the Y-axis giving the position measurements versus the filtered position. This ultimately makes the position throughout the follow by *H. arcticus* smoother. The color bar on the side is an indication of velocity; red represents a faster velocity and blue represents a slower velocity. The last graph shows the head and setae filtered velocity, the X-axis represents time and the Y-axis represents velocity in millimeters per frame.

Number of Follows/Number of encounters	4mm Trail	2mm Trail
H. shoshone	18 follows/76 encounters	5follows/23 encounters

H. arcticus	12 follows/25 encounters	0 follows/4 encounters

Table 1: Shows the number of follows compared to the number of encounters on the 4mm trail compared to the 2mm trail for *H. shoshone* and *H. arcticus*. When compared using a two-tailed t-test the results were significant ($p<0.05$).

Average Angle of Entrance	4mm Trail	2mm Trail
H. shoshone	29.16°	21.08°
H. arcticus	39.69°	NA

Table 2: Shows the average angles of entrance for both species on both trails. When compared using a two-tailed t-test the results were significant ($p<0.05$).

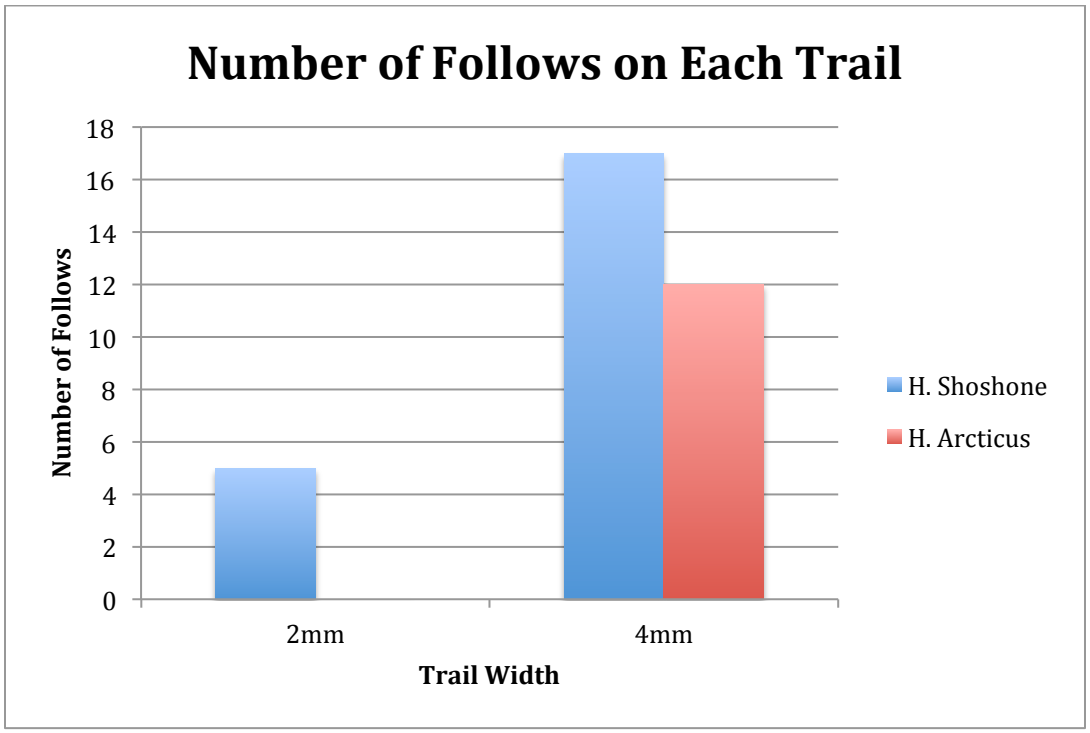


Figure 5: This graph shows the number of follows on the narrow versus wide trail for both species.

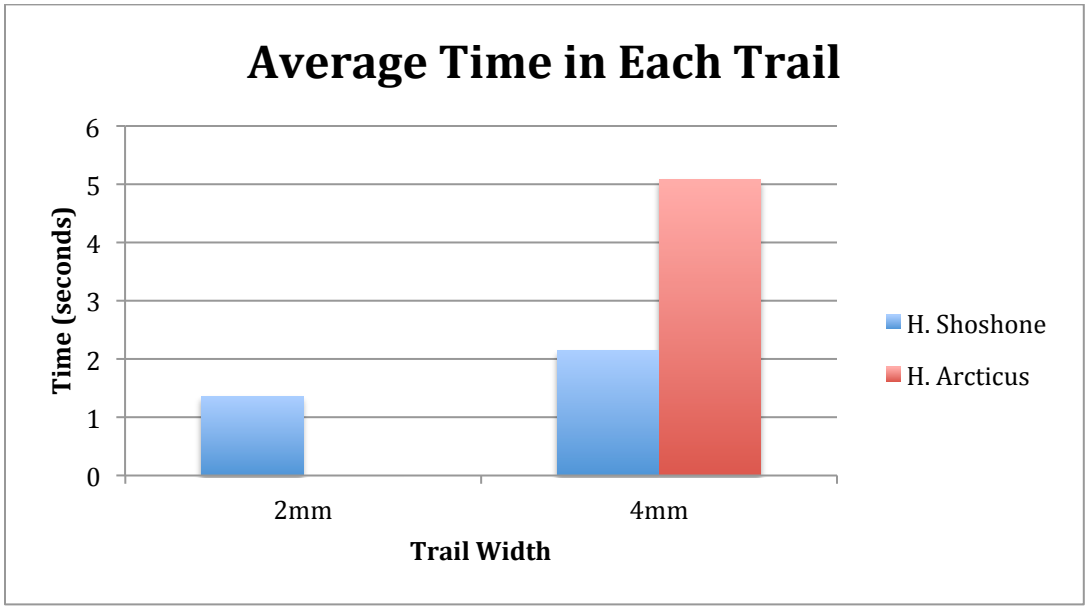


Figure 6: This shows the average time spent in the trail for each species.

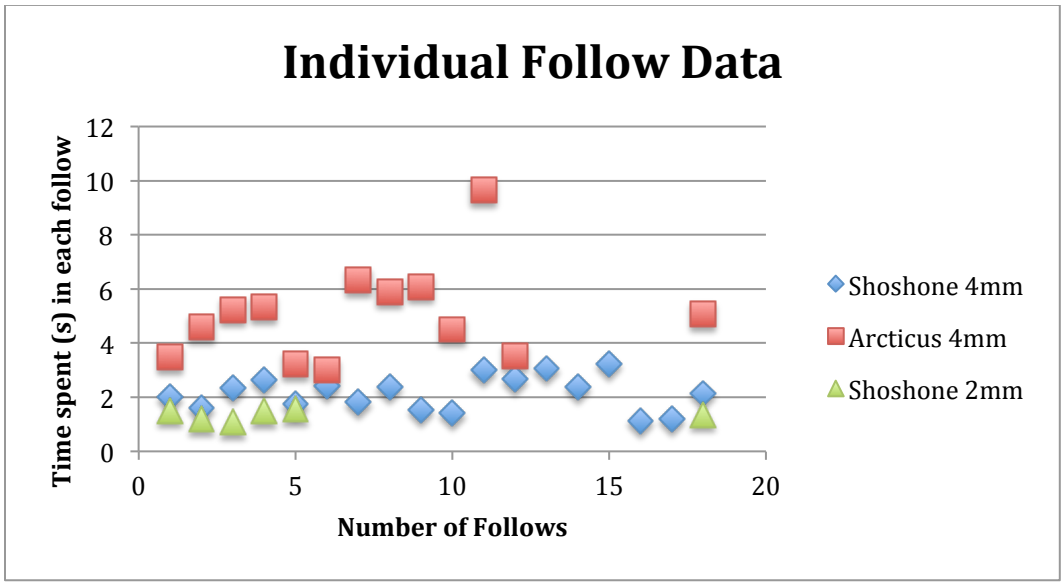


Figure 7: This graph shows the time spent in the trail for each individual follow

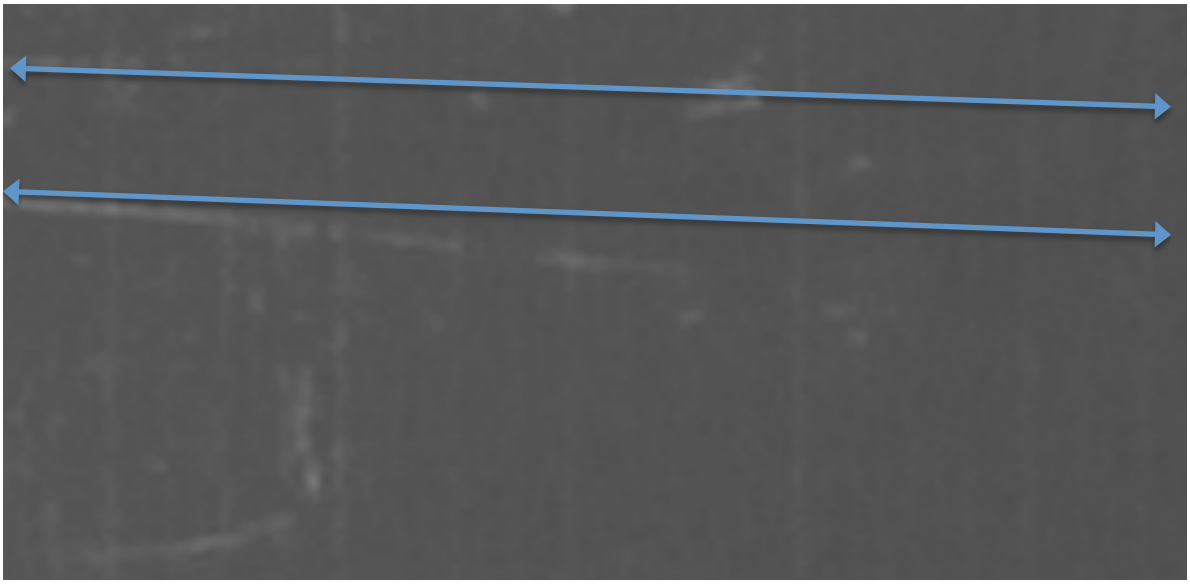


Figure 8: This illustrates an undisturbed trail illustrated by the 3D Schlieren.

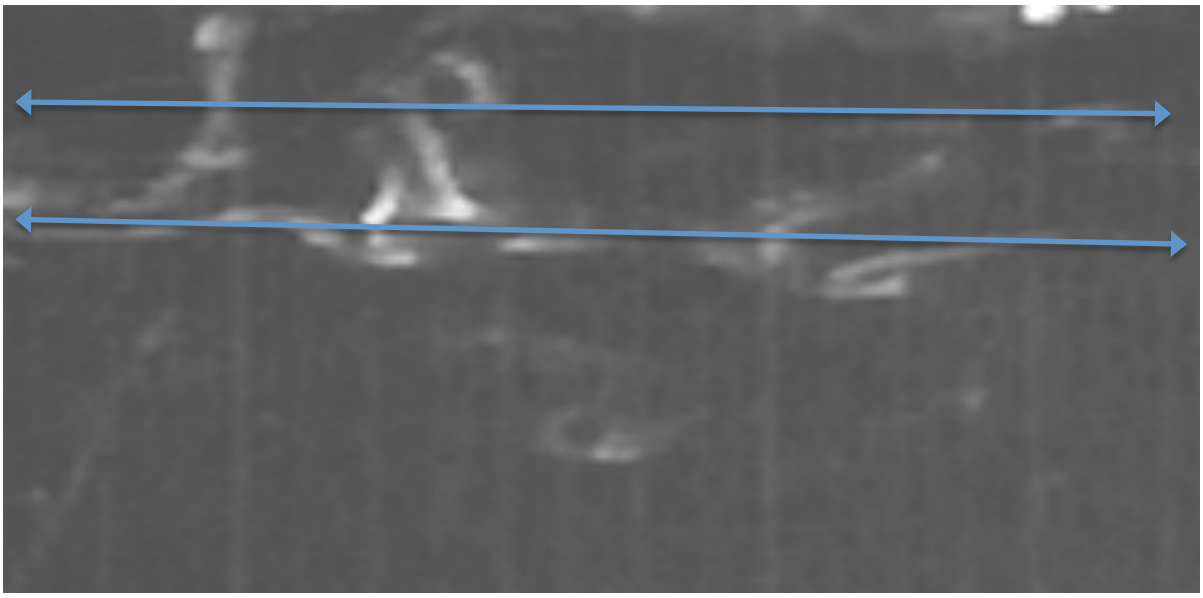


Figure 9: This image depicts a trail immediately after a follow; image is very distorted because of the strong movement of the copepod in the water

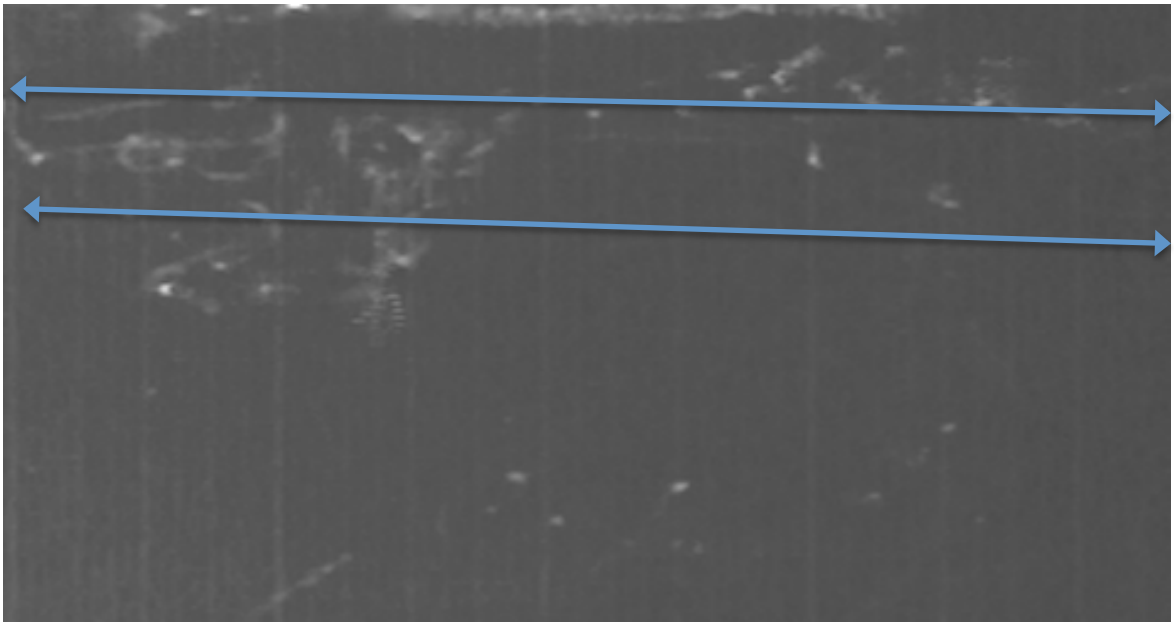
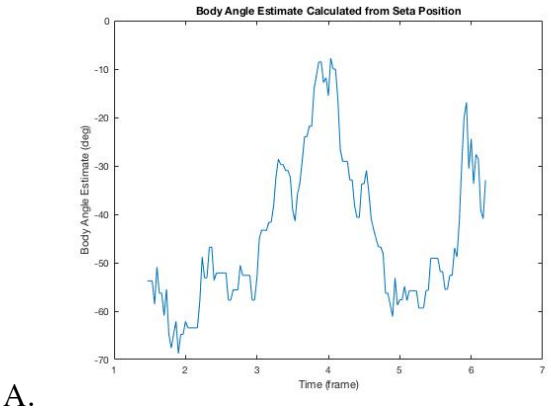
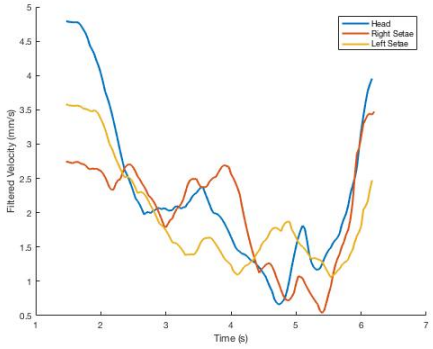


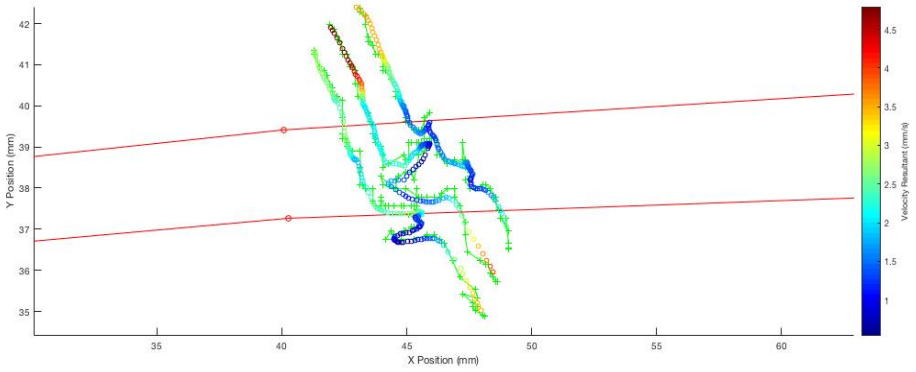
Figure 10: This displays the trail just after an encounter, the trail is distorted but not completely displaced



A.

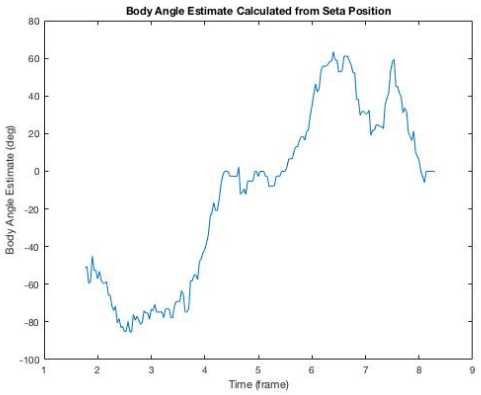


B.

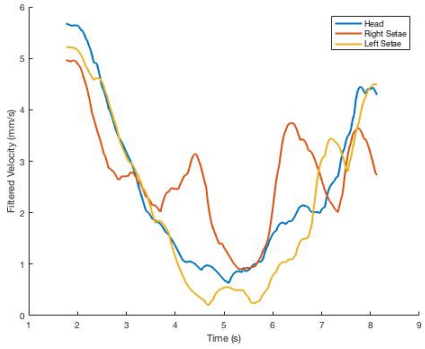


C.

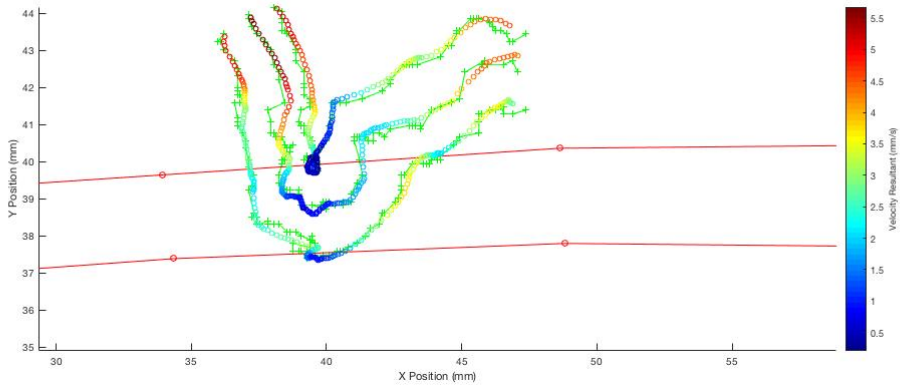
Figure 11: *H. arcticus* spent a lot of time in the middle of the trail and showed great variation in body angle (graph A) due to trying to swim upstream against the high velocity present in the middle of the trail. In graph B it is seen that the velocity is the highest at the beginning and end of a follow, consistent with most behavior seen throughout the experiment. Graph C, the position vs. filtered position graph shows that the copepod exited and entered on different sides and did not swim straight up the trail but struggled to increase its velocity against the strong flow in the middle of the trail. In graph A and B the copepod entered the trail at 2 on the time axis and left at 5.4.



A.

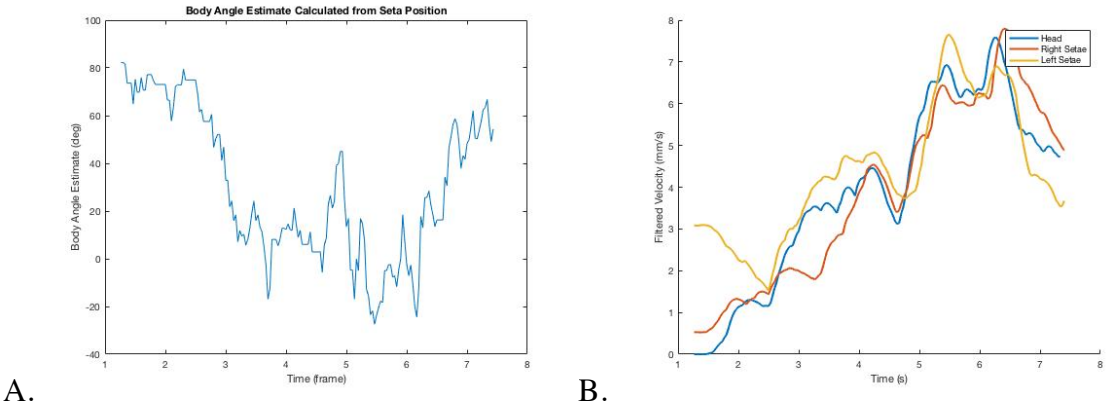


B.

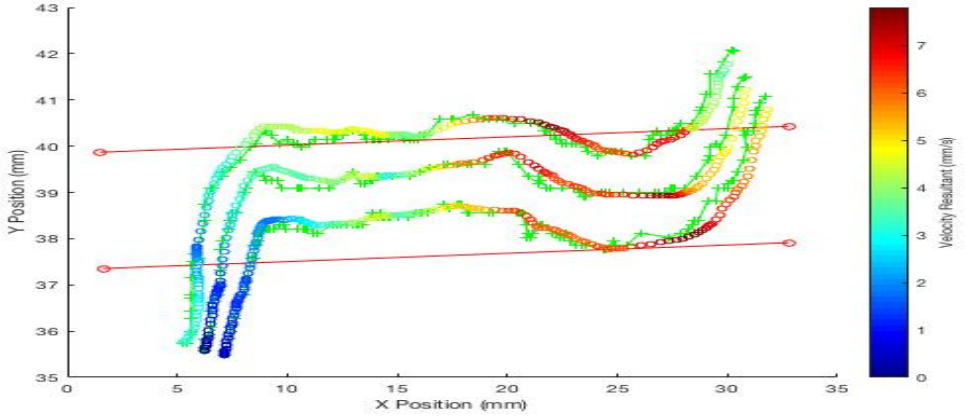


C.

Figure 12: In this follow, the copepod enters the trail at 2 on the time axis and leaves at 6 in graphs A and B. Graph A shows that the animal changed its body angle in a consistent manner through the second half of the follow as it was rotating to exit the trail, as seen by the blue color on graph C. The copepod enters and exits on the same side of the trail and exhibits a relatively smooth trajectory through the trail. The path is much cleaner than that in figure 11 but still increases in velocity as the follow ends. Graph B shows that the velocity is highest at the beginning and end.

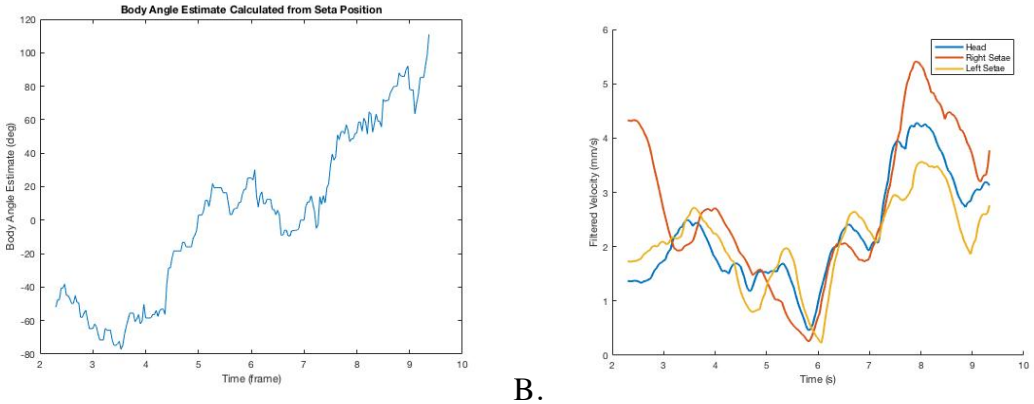


A. B.

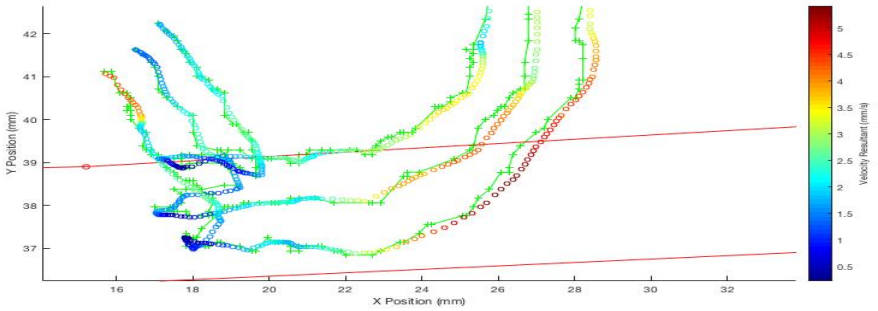


C.

Figure 13: The copepod enters the trail at 2.5 and leaves at 6.8 on the time axis in graphs A and B. Graph A shows that there is a lot of variation in body angle even though the track is smooth, but because the copepod is following the middle of the trail (graph C), it is fighting the flow as it is swimming. The copepod enters and exits on different sides of the trail (graph C) and increases its velocity as it is exiting the trail as seen by the peaks in graph B.

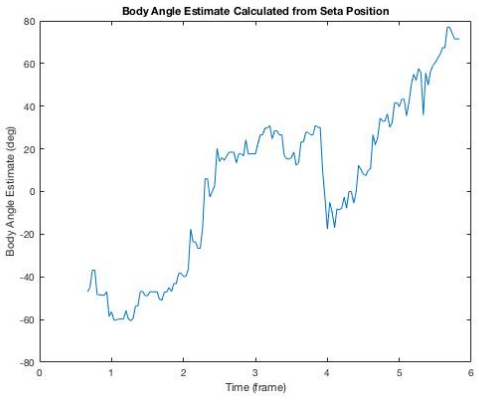


A. B.

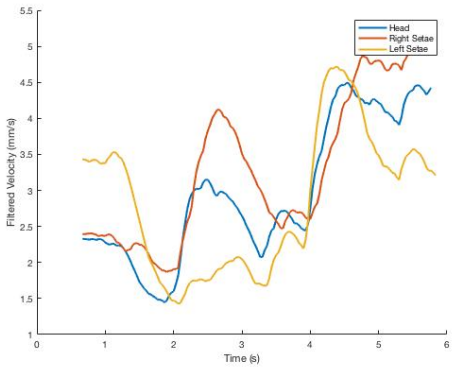


C.

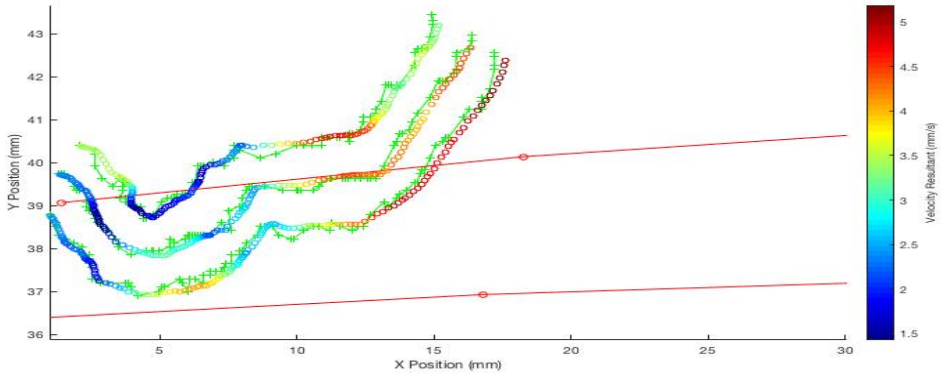
Figure 14: In this follow, the copepod enters the trail at 2 on the time axis and leaves at 8 in graphs A and B. This follow is very similar to figure 12, the copepod enters and exits on the same side of the trail and exhibits a relatively smooth trajectory and body angle through the trail (graph A). In this instance the copepod stays in the trail for a longer period of time and starts out with a slower initial velocity, but leaves with a high velocity as seen in many other instances (graphs B and C).



A.



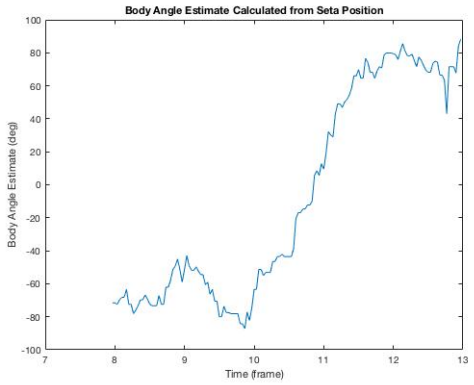
B.



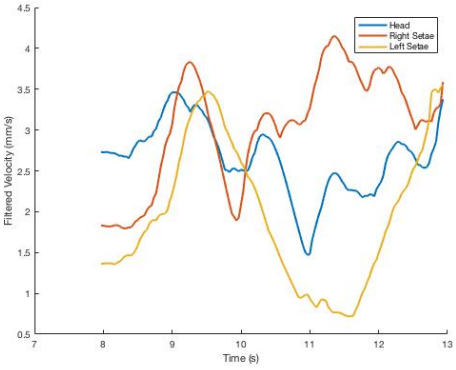
C.

Figure 15: In this follow, the copepod enters the trail at 0.5 on the time axis and leaves at 4.5 in graphs A and B. This follow is similar to figure 14 except the copepod spent less time in the trail and the velocity is higher throughout (graph C). This is because the animal stays closer to the edges of the trail where the flow is weaker and the copepod can

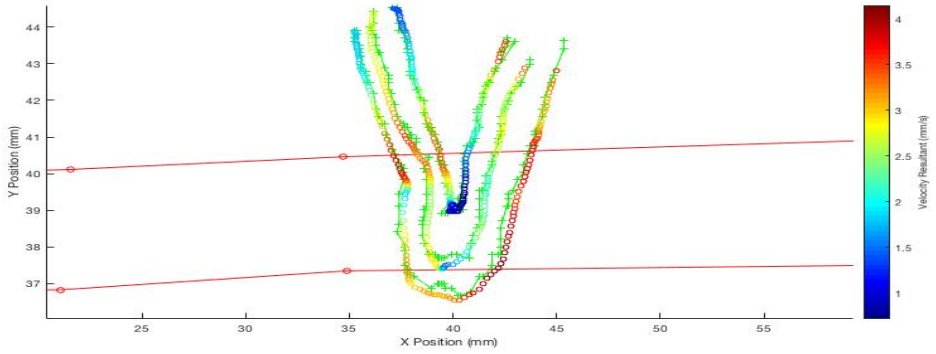
swim upstream faster (graph C). In graph A the body angle pattern is similar to other follows (graph A) and in graph B the velocity is highest at the end of the follow.



A.

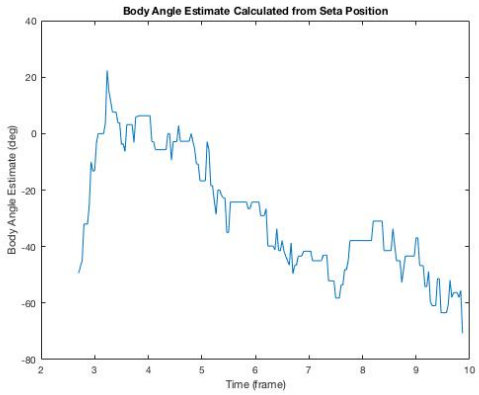


B.

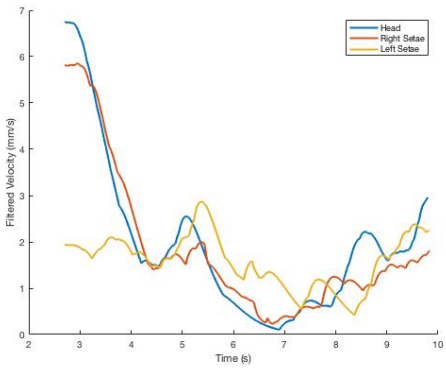


C.

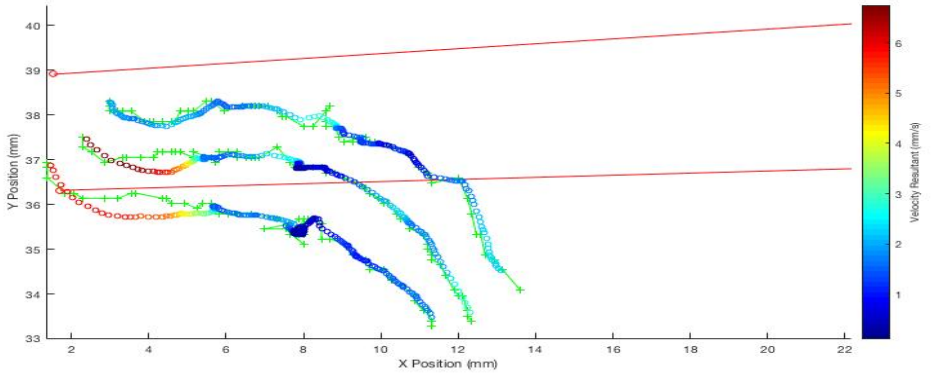
Figure 16: In this follow, the copepod enters the trail at 9 on the time axis and leaves at 12 in graphs A and B. This follow is a slight outlier because even though the copepod enters and exists on the same side of the trail, it takes him longer to swim over to the other side and back again (graph C). The velocity on the left setae is very high throughout because the copepod rotates around the left setae during the whole follow (graph B). In graph A the body angle patter in similar to figure 15.



A.

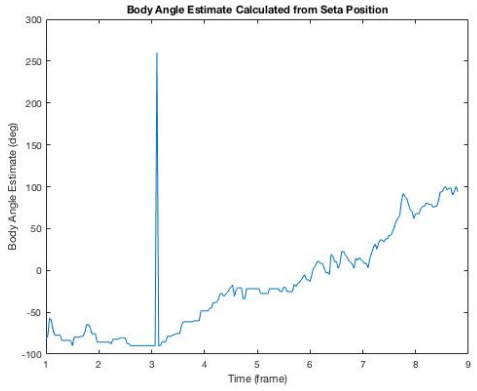


B.

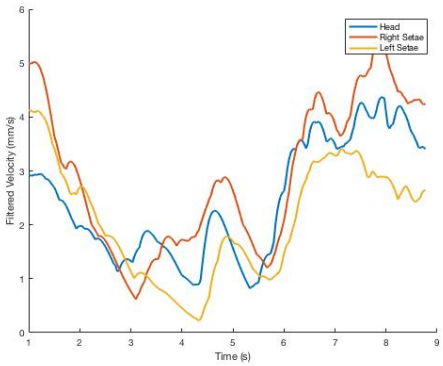


C.

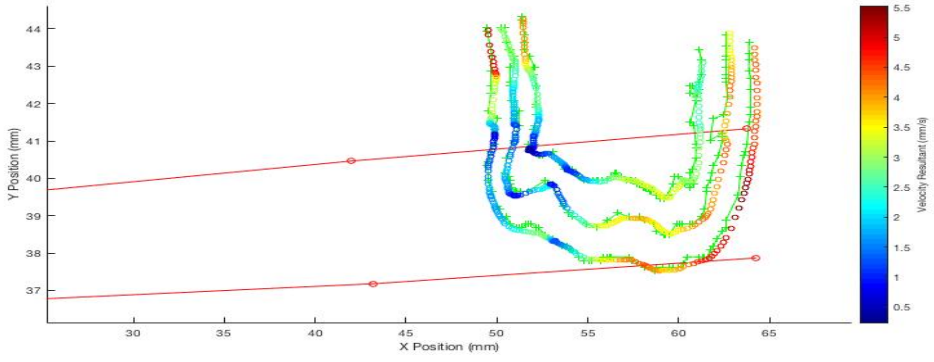
Figure 17: In this follow, the copepod enters the trail at 2 on the time axis and leaves at 9 in graphs A and B. This follow is similar for figure 11 except that the velocity is the highest as the copepod is entering the trail as opposed to exiting it (graphs B and C). As the copepod is leaving the trail, it makes some position adjustments as seen by the dark blue color in graph C. the position adjustments can also be seen by the large variation in body angle in graph A. Additionally, the velocity is low at the end which is different from most other follows (graph B).



A.



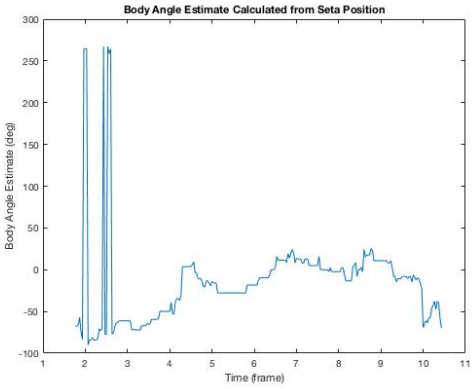
B.



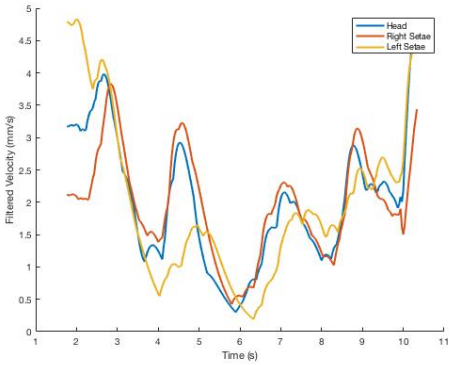
C.

Figure 18: In this follow, the copepod enters the trail at 3 on the time axis and leaves at 8 in graphs A and B. Similar to figure 14, the copepod enters and exits on the same side of

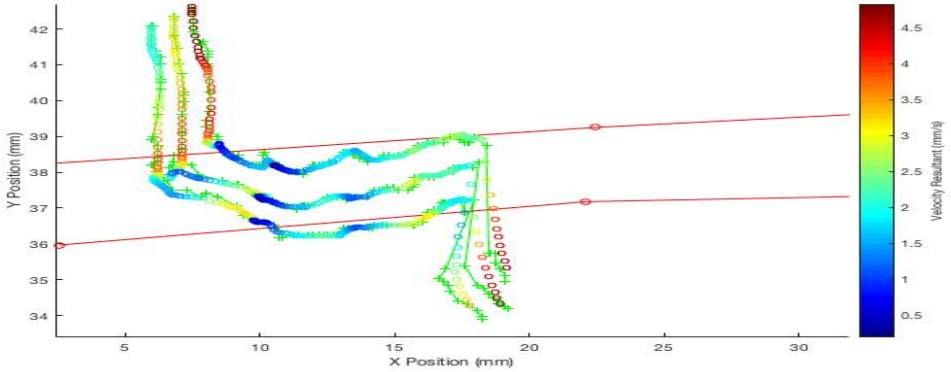
the trail and exhibits a relatively smooth trajectory through the trail. The copepod must reorient itself in some way as it is entering the trail depicted by the large peak in graph A. the velocity pattern in graph B is similar to other follows with high velocity at the beginning and end. In this instance the copepod stays in the trail for a longer period of time and starts out with a slower initial velocity (graph C).



A.

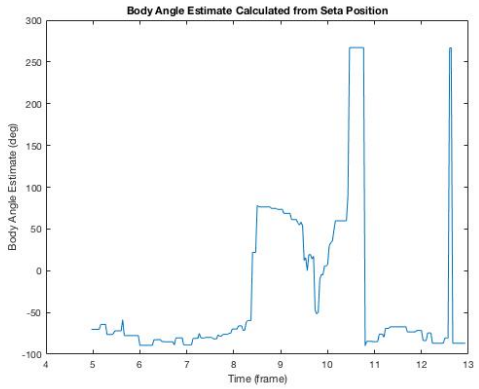


B.

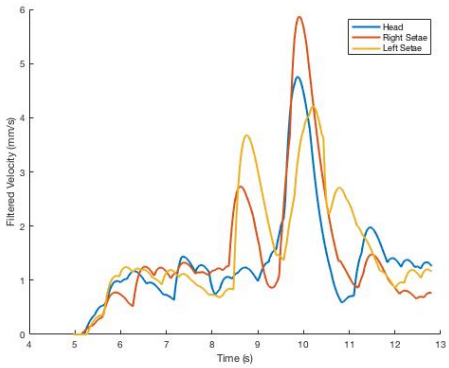


C.

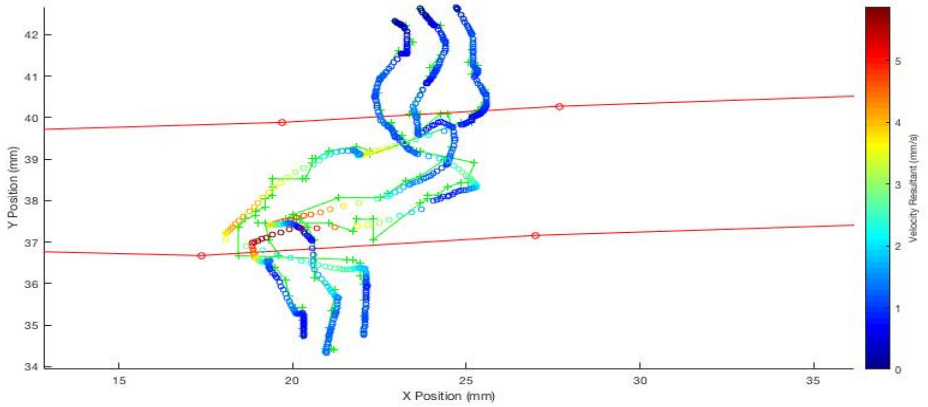
Figure 19: In this follow, the copepod enters the trail at 3 on the time axis and leaves at 9 in graphs A and B. This follow is similar to figure 13, but there is less variation in body angle once the copepod has entered the trail (graph A) and the velocity slows down and speeds up more frequently and not just at the beginning and end of the follow (graphs B and C).



A.

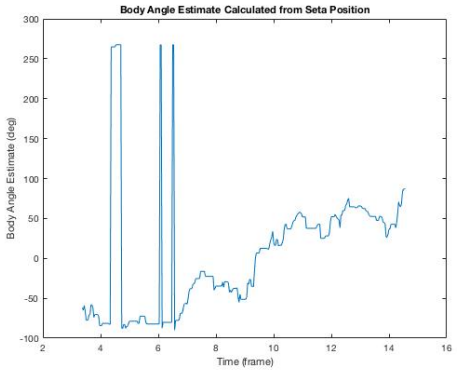


B.

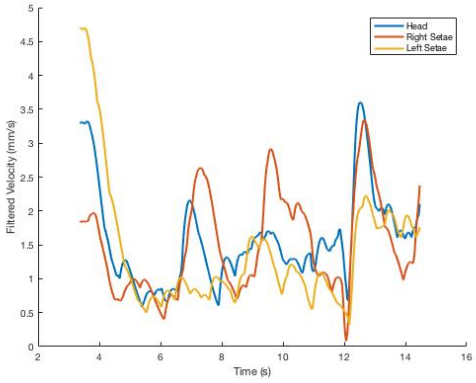


C.

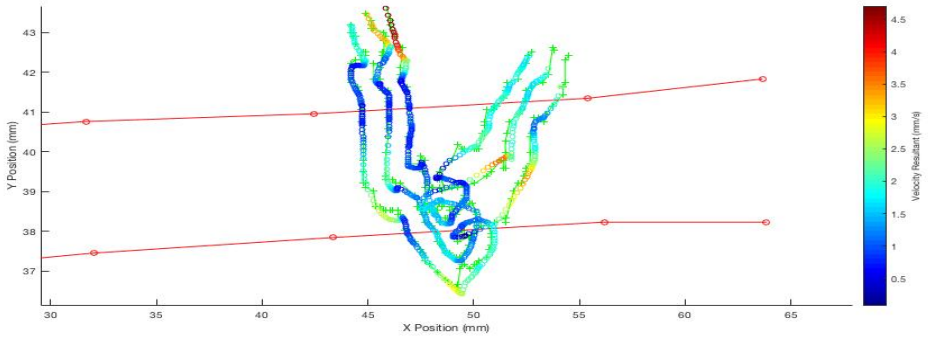
Figure 20: In this follow, the copepod enters the trail at 8 on the time axis and leaves at 11 in graphs A and B. This follow is also abnormal compared to the others. The body angle of the copepod depicted in graph A shows sharp peaks while the copepod is in the trial. The copepod enters and exists on different sides of the trail and has high velocity in the middle of the follow unlike the other follows (graphs B and C). Additionally this was the longest follow, about 10 seconds.



A.

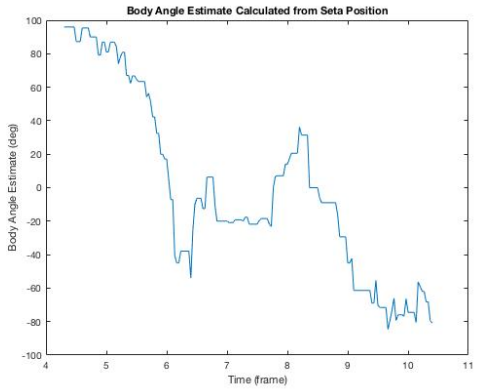


B.

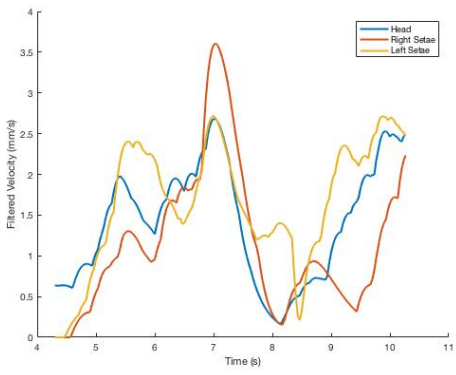


C.

Figure 21: In this follow, the copepod enters the trail at 4.5 on the time axis and leaves at 12 in graphs A and B. Similar to figure 18, the copepod enters and exits on the same side of the trail and exhibits a relatively smooth trajectory through the trail except for position adjustments as it is exiting (graph A). In this instance the copepod stays in the trail for a longer period of time and starts out with a slower initial velocity but quickly increases its' velocity and keeps it higher throughout compared to other follows (graph B and C).



A.



B.

C.

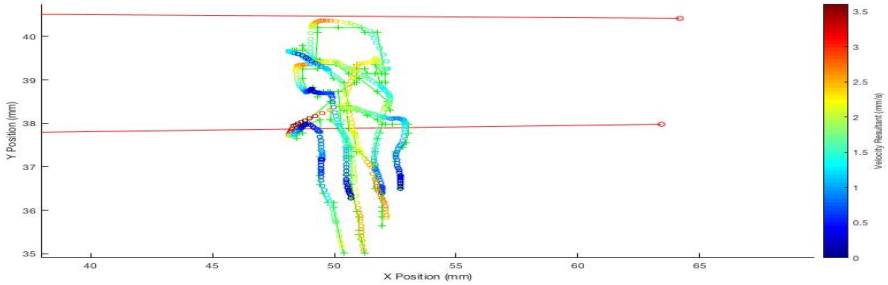


Figure 22: In this follow, the copepod enters the trail at 5.5 on the time axis and leaves at 9.5 in graphs A and B. This is a slightly different swimming pattern because the copepod enters the trail, leaves, re-enters, and exits on the same side, which is seen in graph A by the peaks and graph C showing the position of the copepod relative to the trail. The velocity peaks as the copepod enters the second time and leaves the second time (graphs B and C), which is consistent with patterns seen throughout the experiment.

CHAPTER 5

DISCUSSION

The goal of this experiment was to compare the behavior of *H. shoshone* and *H. arcticus* in planar dextran trails, and ultimately find Strouhal's number for both species. Part of the hypothesis was supported because both species prefer to follow the wider 4mm trail, and *H. arcticus* spend more time in the trial than *H. shoshone*. Another interesting observation revealed that *H. arcticus* spend more time in the middle of the trail and show obscure behavior during a follow (figures 11-22). As seen in the figures no two graphs are exactly alike indicating great variation in swimming behavior. Sometimes they will clearly disturb the trail so it can be considered a follow, but they will simply not ascend the trail with a similar speed. This could be due to a size discrepancy or because they use the setae on their antennae in a different way. Changing the flow rate of the trial might alter this behavior. Additionally, *H. arcticus* show great variation in behavior. Their movements include swimming down while keeping their orientation facing upward, moving in a circular motion while in the trail or rotating, and following the trail directly up the middle. Based upon observation of the two species it seems that *H. shoshone* want to reach their end goal, food or a mate, while *H. arcticus* show variation and potentially take more time to detect disturbances or get disoriented once in the trail.

Only *H. arcticus* was analyzed using the MatLab computer program that computes the X and Y positions of the head and setae, body angle estimated from the setae location, body angle rate estimate, measure of position versus filtered position, and filtered velocity of the head and setae (Figure 11-22). These data showed some interesting results. In general the copepods increase their velocity when they are entering

and exiting a trail, which indicates that their reflex sense in response to changes in the environment is strong. The filtered velocity figures confirm this. However, once inside the trail the velocity slows and the body angle varies greatly in all follows. The X and Y positions show some consistency in position in the trail, but more trials will need to be run in order to determine if they are statistically significant results. Most of the time the copepod enters the trail, crosses over to the other side, and then comes back to the side of entrances and exits. The orientation of the antennae in the trail are represented by the X and Y graphs, and this means that the position of the antennae in the trail is what stays consistent. In this experiment there were not eight follows that were similar enough, due to variation in swimming patterns, and that is the number needed for significant power in statistical tests. However, there were eight separate replicates done which is enough for power analysis.

The graphs that are "filtered" simply mean that the data was manipulated so that all tracked points were taken into account and the smoothest path was graphed. In some cases there were points that were outliers, so the program removed these to generate more consistent data.

The angle of entrance was computed for both species and the results show, with statistical significance, that *H. arcticus* enter the trail at a greater angle (39.69°) than *H. shoshone* on both trails (29.16° on the 4mm, and 21.08 on the 2mm). This information was obtained using data points from *Hendrick's.* One explanation for the large difference between the two species could be that *H. shoshone* senses the trail with setae in a different location on their antennae than *H. arcticus*. Size difference between the two species and the difference in the span of the antennae is a probable explanation for this

behavior. A greater angle of entrance could indicate that *H. arcticus* have main sensory setae closer to their head than *H. shoshone*. It is thought that this is the case because *H. arcticus* insert themselves farther into the trail before they follow it. It can be argued that since *H. shoshone* enter at a narrower angle, they are able to pick up on different hydrodynamic cues with more efficiency than *H. arcticus*. This speculation was made based on the closer to the end of the antennae the sensor is, the wider their detection range is which is helpful is location food and mates. The result of a difference in size is that the separation of sensory setae along the antennule changes. For a smaller copepod, the setae are more closely spaced and smaller hydrodynamic cues can be detected. The increased movement of the smaller copepods may indicate it is detecting finer fluid deformations in the trail. This hypothesis is consistent with the findings of this experiment. The smaller copepods had to insert themselves into the trail further with a greater angle, but were able to respond faster once they were in the trail. The larger copepods found the trail quickly and with very little reorientation and a small angle of entrance, however the follows exhibited by them were short.

In contrast to the behavior *of H. arcticus,* in all follows by *H. shoshone*, once in the trail they simply swim upstream quickly without much variation. In some occurrences they will enter on one side of the trail, cross over to the other edge, and exit. This behavior may indicate that they prefer to follow the edges of trail because of the density difference between the dextran trail and EPA water. Given this information more experiments could be run with even wider trails to determine if *H. shoshone* primarily follow the edges. If the copepods sense the edges of the trail, that indicates they are sensing sheer. Following the middle indicates they prefer faster speeds. Ultimately, from

this study *H. Shoshone* sense sheer and *H. Arcticus* prefer faster speeds. With these data further studies may determine which setae on the antennae are used in the detection of different disturbances within their environment and explain why each copepod prefers edges or the middle of the trail.

In theory, the smaller copepod should be able to detect smaller signals in the environment which is a probably explanation for why the body angle graph shows such variation throughout the follow in all twelve events. The copepod may be picking up on small signals in the middle of the trail that cannot be seen on the videos, and reacting to them quickly in order to continue swimming upstream. Changing the flow rate or adding other small disturbances and repeating the experiments could help confirm that *H. arcticus* detect finer details in their environment and are able to respond more quickly than *H. shoshone.*

Throughout the experiment there are a few places where discrepancies were found. Firstly, some of the experiments were run in the morning and some in the evening. Time is not a known factor affecting the behavior *H. shoshone* or *H. arcticus*, but the time they were fed with relation to the experiment could be a factor. Additionally, throughout the experimental process, caution was taken to ensure that the pH and temperature were consistent with the natural habitat of the copepods, but there could still be variations that potentially affect the results of the experiments. Ultimately in order to verify that the results are as accurate as possible it is preferable to run more replicates.

Preliminary observations evince that the copepods exhibit a variety of behaviors in the wide trail, sometimes they swim along only one side, sometimes *H.arcticus* move

side to side showing the wobbling behavior, and occasionally they follow one side, swim to the other side, and then continue following on the other side.

The path a copepod takes once in the trail may vary between species and different follows, but the orientation at which the copepod enters the trail always remains constant. The copepod always reorients itself to be in plane with the trail, never sideways. Another consistency found in both species is the way they travel once in the trail. They always follow upstream with their head above their tail. An additional regularity is that both species follow a potential female scent, and try to arrive at this destination soon as possible.

CHAPTER 6

FUTURE WORK

The results of this experiment provide preliminary information on how two different species of copepods within the same genus interact with planar dextran trails. The goal of this study was to determine Strouhal's number for the animals, but this could not be done because the trails were too short, there was not enough area covered by the camera to get a frequency. In the future using only *H. arcticus* to experiment with Strouhal's number calculations could be beneficial. Experimenting with different flow rates could help normalize the swimming behavior and potentially afford enough information to produce a frequency within the trail. From these data obtained from MatLab there are great variations in the movement *H. arcticus* exhibit in the trail, and running more replicates will solidify preliminary observations. The other components of Strouhal's number can be found with the date from these experiments, the only missing component is the frequency. *Hesperodiaptomus arcticus* appear to have sensory setae closer to their head than *H. shoshone*. This prediction was made based upon the greater angle of entrance observed in *H. arcticus* and that they are smaller and thus have setae that are closer together. The assumption made here imparts that the copepod must insert

itself farther into the trail before it senses that there is a hydrodynamic signal it should respond to. Additionally, since this species prefers the high speed in the middle of the trail, it could insert itself farther into the trail before committing to a follow in order to find the faster speed. This could indicate that *H. arcticus* are less sensitive to hydrodynamic cues than *H. shoshone*. To further confirm this theory, experiments should be conducted with a high-magnification system. The system used in this experimental procedure is a low-magnification system and does not expose as much detail. The outline of the copepod and its antennae can be seen in relation to the trail, but in a high magnification system the lighting and cameras produce more detailed images. The individual setae can be observed in the high-magnification system, which is a component that could be used to confirm that *H. arcticus* have sensory setae closer to their head than *H. shoshone*. Running the MatLab program on follows from *H. shoshone* would also be beneficial in determining the location of the main sensor on the setae. This is a step that should be done in future experiments.

Hesperodiaptomous arcticus show varying behavior that is hard to quantify, and *H. shoshone* show very basic behavior. They sense a trail, follow it, and leave. This behavior demonstrates that they are efficient in locating mates and food, as well as escaping predators. Future work conducted on this topic could experiment with varying flow rates and even wider trails to produce a frequency in swimming behavior. The results of this experiment clearly show that both species prefer the larger trails, and therefore there is no need for further experimentation with trails less than 4mm in width if finding Strouhal's number is the end goal. These data collected during this experiment

can also be further analyzed to find the velocity of the copepod before, during, and after following the trail.

REFERENCES

Amos, W (n. d.) Behavioural Ecology. Retrieved November 30, 2016, from http://www.zoo.cam.ac.uk/directory/research-themes/behav-ecol

Fischer, J. M., Olson, M. H., Williamson, C. E., Everhart, J. C., Hogan, P. J., Mack, J. A., Vinebrooke, R. D. (2011). Implications of climate change for daphnia in alpine lakes: Predictions from long-term dynamics, spatial distribution, and a short-term experiment. *Hydrobiologia, 676*(1), 263-277

Frederick, J. (2015). Behavior of *Hesperodiaptomus shoshone*: Hydrodynamic cues of Trail Width. *Georgia Institute of Technology School of Biology, 1*(1), 1-20.

Kiorboe, T., Andersen, A., Langlois, V. J., & Jakobsen, H. H. (2010). Unsteady motion: Escape jumps in planktonic copepods, their kinematics and energetics. *Journal of The Royal Society Interface, 7*(52), 1591-1602.

Marszalek, M. A., Dayanandan, S., & Maly, E. J. (2009). Phylogeny of the genus *Hesperodiaptomus* (copepoda) based on nucleotide sequence data of the nuclear ribosomal gene. *Hydrobiologia, 624*(1), 61-69.

Pender-Healy, L. (2014). Tracking response of the freshwater copepod *Hesperodiaptomus shoshone*: Importance of hydrodynamic features. *Georgia Institute of Technology School of Biology* ,1, 1-27.

Rautio, M., & Vincent, W. F. (2006). Benthic and pelagic food resources for zooplankton in shallow high-latitude lakes and ponds. *Freshwater Biology, 51*(6), 1038-1052.

Reid, J.W., and C.E. Williamson. 2010. *Copepoda*. In: Thorp, J.H. & A.P. Covich (Eds.). Ecology and Classification of North American Freshwater Invertebrates. 3rd Edition. Chapter 21. Academic Press, New York: 829-899.

Taylor, G. K., Nudds, R. L., & Thomas, A. L. R. (2003). Flying and swimming animals cruise at a Strouhal number tuned for high power efficiency. *Nature, 425*(6959), 707-11. Retrieved from http://prx.library.gatech.edu/login?

Taylor, J. (2016). Finding Food in an Aquatic Desert: How cruising copepods detect their next phytoplankton meal. *Georgia Institute of Technology School of Biology, 1*, 1-25.

Wang, S., & Ardekani, A. M. (2012). Unsteady swimming of small organisms. *Journal of Fluid Mechanics, 702*, 286-297. doi:10.1017/jfm.2012.177

Yen, J., Sehn, J. K., Catton, K., Kramer, A., & Sarnelle, O. (2011). Pheromone trail following in three dimensions by the freshwater copepod *Hesperodiaptomus shoshone*. *Journal of Plankton Research, 33*(6), 907-916. doi:10.1093/plankt/fbq164